Analytical Methods and Composition of Fatty Materials

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KNOWLEDGE of the composition of fats and industrial fatty materials is very important in nearly every phase of fat chemistry and technology although often its importance is not fully realized. In fact, progress in the utilization of commercial fats and fatty acids as raw materials in the manufacture of industrially useful products is dependent to a large extent on knowledge of the composition of the starting material and of fractions derived from it. Numerous examples could be cited in both inedible and edible fat technology where such information is indispensable. Indeed it is difficult to cite any commercial process for utilizing fatty materials in which compositional factors are not essential. Methods for determining composition, of course, should be equally important.

It is obviously impossible in this limited space to treat so broad a subject in a detailed and comprehensive manner or to relate the history and development of the methods. An attempt has been made only to discuss the essential features and applications of analytical methods for determining fatty acid and glyceride composition.

Fatty Acid Composition

The procedures or methods to employ in determining fatty acid composition depend upon several factors: a) how complete information is needed, b) the type of fat or fatty material, c) prior treatment and condition of the material, and d) the amount of sample available. Furthermore it is often necessary to separate complex fats and fatty acids into a number of simpler fractions before applying analytical methods. Hence it would hardly be possible to outline procedures which would apply equally well to all fatty materials.

Chemical Constants. In general, it is common practice to characterize the sample by determining its chemical and physical constants. The constants often determined are iodine, saponification and thiocyanogen numbers, refractive index, unsaponifiable matter, titer, melting point, moisture, color, etc. Each of these constants is of definite value in describing the material, and some at least give qualitative information related to composition. The first four constants mentioned, particularly when they are used in conjunction with other analytical data or applied to simple mixtures, are of value in quantitative estimations of fatty acid composition. The Wijs method appears to be most widely used for determining iodine numbers of our most common fats and oils, which contain little or no conjugated acids. The Benham-Klee method (2, 26, 42, 48), and methods based on hydrogen uptake (39, 40, 49, 52) are better suited for fats containing conjugated acids. The iodine number is a measure of total unsaturation and without supplementary information does not define the specific unsaturated acids present. However in simple mixtures, such as may be obtained in fractional distillation of fatty acids or methyl esters, it may be used for calculating composition, providing the mixture contains only two known unsaturated components, or two known unsaturated and a known percentage of saturated components (determined independently). An example may be cited. A fraction of methyl esters, iodine number 105, contained 10% saturated esters (by Bertram method [3]); methyl oleate and linoleate were the only unsaturated esters:

$$I_u = \frac{100\, I_s}{100 - S}; \quad \frac{I_u - I_1}{I_2 - I_1}(100 - S) = \% \text{ methyl linoleate}$$

$$\frac{I_2 - I_u}{I_2 - I_1}(100 - S) = \% \text{ methyl oleate}$$

where I_u = iodine number of unsaturated components of sample
I_s = iodine number of sample
I_1 and I_2 = theory iodine numbers of methyl oleate and linoleate
S = % saturated esters

Calc.: I_u = 116.7; % methyl oleate = 57.8; % methyl linoleate = 32.2.

Similarly, if the percentage of either unsaturated ester is known or determined independently, the proportion of the other unsaturated and the saturated esters can be calculated. For example, if the methyl linoleate is determined spectrophotometrically:

$$\frac{100\, I_s - L I_2}{I_1} = \% \text{ methyl oleate, where } L = \% \text{ methyl linoleate}$$

The saturated esters are obtained by difference.

Thiocyanogen numbers (19, 37, 47) may be used in conjunction with iodine numbers to estimate the percentages of oleic, linoleic, and saturated acids (or esters) in fats or fractions containing only these components. If linolenic acid is also present, the percentage of each unsaturated component can be calculated, providing the percentage of saturated is determined independently. The thiocyanometric method to a large extent has been superseded by spectrophotometric methods.

The saponification number also serves a useful purpose in composition studies. It is the number of milligrams of KOH consumed in the saponification of 1 g. of sample. By dividing the equivalent weight of KOH in milligrams by the saponification number, the saponification equivalent is obtained. It is a measure of average chain length or mean molecular weight for normal fatty acid esters and is particularly valuable in connection with analyses of fractions obtained in fractional distillation or other means of separation of multi-component ester mixtures. When working with fatty acids, the neutralization number or equivalent gives the same type of information. For simple binary mixtures of ester or acid homologues, the proportion of each can be estimated.

$$\frac{N_s - N_B}{N_A - N_B} \times 100 = \% \text{ A}; \quad 100 - \% \text{ A} = \% \text{ B}$$

where N_s, N_A, and N_B = saponification or neutralization numbers of the sample, and components A and

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B, respectively. Inherently this method of estimating composition is not one of high precision owing to the relatively small differences between saponification or neutralization numbers of homologues and to the probable error in determining these numbers.

The refractive index can be used similarly to estimate the composition of binary mixtures, when the index for each pure component is known, and is useful as a guide in changing fractions in distillations.

Ultraviolet Spectrophotometric Methods. The polyunsaturated acids present in most of our common fats and oils are non-conjugated and show no absorption in the near ultraviolet above 200 mμ. These acids can be rearranged by simple treatment with alkali at high temperatures to conjugated isomers, which have well-separated spectra in the readily accessible u.v. region (200-400 mμ). Thus a simple treatment with alkali has formed the basis for our most important method of analysis of fats and oils (7, 25, 31, 32, 34). The rearrangement of double bonds (prototropic shift) occurring in this reaction may be represented as follows, where (I) = linoleic acid pentadiene system:

$$\underset{(I)}{R-CH=CH-CH_2-CH=CH-R'} \xrightarrow[\text{alkali}]{\Delta}$$

$$\left.\begin{array}{l}(R-CH=CH-\overline{C}H-CH=CH-R'\,[II]\;)\\(R-\overline{C}H-CH=CH-CH=CH-R'\,[III])\\(R-CH=CH-CH=CH-\overline{C}H-R'\,[IV]\;)\end{array}\right\} + H^+$$

Owing to the lability of the hydrogen atoms on the middle carbon of (I), a proton is removed by ionization to form the unstable negative ion (II), which immediately undergoes resonance stabilization to the conjugated resonating ions (III) and (IV). Since the double bonds are far removed from the influence of the carboxyl group, one would expect an equal amount of 9,11- and 10,12-conjugated dienoic acids to be produced from linoleic acid. From considerations of the mechanism of the prototropic shift and probable spatial conflicts involved in the formation of certain isomers, one would also expect that the double bond which shifts would assume the *trans*-configuration. Evidence in support of these views has been published (33, 21).

When non-conjugated acids containing more than two double bonds are isomerized with alkali, *e.g.*, linolenic acid, the same type of prototropic shift takes place, but a mixture of isomers is formed in which not all of the double bonds in each molecule are in conjugated position. Linolenic acid (V) has two active methylenes and may be considered as undergoing a 2-stage prototropic shift. The following isomers, VI, VII, VIII, and IX, would be formed in the first stage.

	16 15 14 13 12 11 10 9	
(Linolenic acid V)	$R-CH=CH-CH_2-CH=CH-CH_2-CH=CH-R'$	
(VI)	$R-CH_2-CH=CH-CH=CH-CH_2-CH=CH-R'$	$\longrightarrow$ triene (conj.)
(VII)	$R-CH=CH-CH=CH-CH_2-CH_2-CH=CH-R'$	$\longrightarrow$ diene (conj.)
(VIII)	$R-CH=CH-CH_2-CH_2-CH=CH-CH=CH-R'$	$\longrightarrow$ diene (conj.)
(IX)	$R-CH=CH-CH_2-CH=CH-CH=CH-CH_2-R'$	$\longrightarrow$ triene (conj.)

Structures VI and IX are capable of undergoing a second stage shift, owing to the remaining active methylene, and would result in triene conjugated isomers. Structures VII and VIII however have an

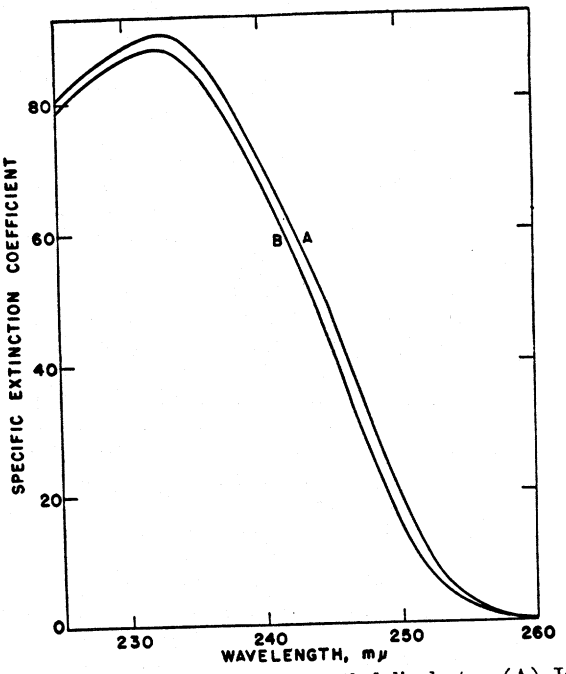

FIG. 1. Absorption spectra of methyl linoleate: (A) Isom erized at 180°C. for 45 minutes in 11% KOH-glycerol unde nitrogen; (B) isomerized at 180° for 15 minutes in 21% KOH glycol under nitrogen.

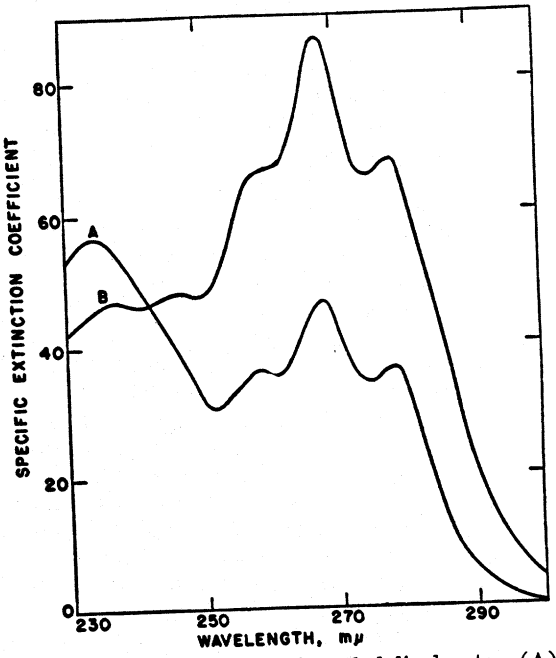

FIG. 2. Absorption spectra of methyl linolenate: (A) (B) same as for Fig. 1.

isolated double bond separated from the other conjugated double bonds by two methylenes, wh are much less labile and consequently would be likely to undergo further prototropism. Hence and VIII would show an absorption maximum in same spectral region as isomerized (conjugated) li leic acid.

Ultraviolet absorption curves for alkali-isomer linoleic, linolenic, arachidonic, and eicosapentae acids are shown in Figures 1, 2, 3, and 4. Each of conjugated acids have three principal absorp maxima, but some of the peaks are obscured by o

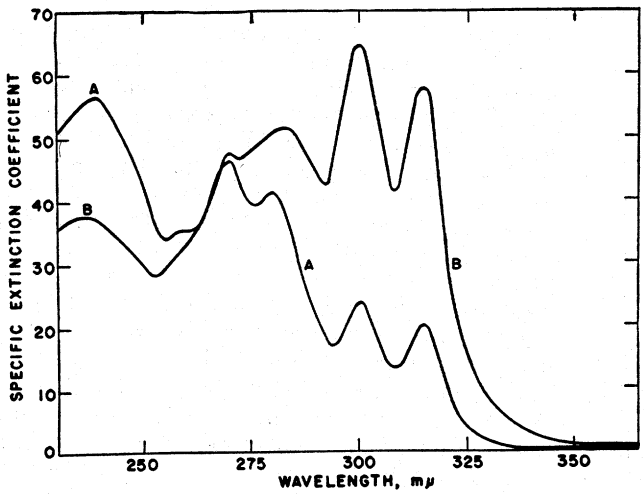

FIG. 3. Absorption spectra of methyl arachidonate: (A) and (B) same as for Fig. 1.

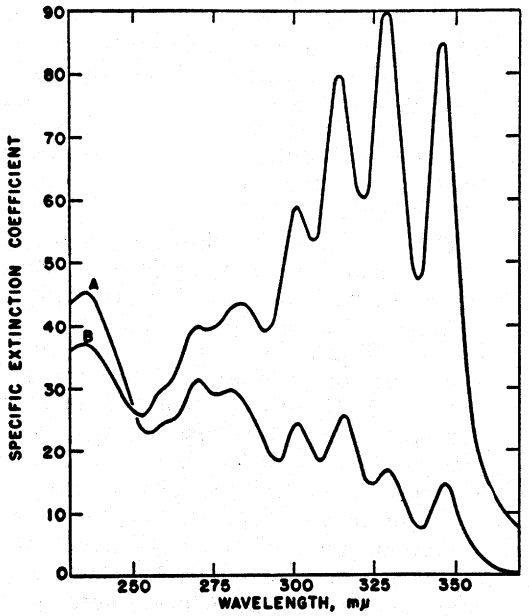

FIG. 4. Absorption spectra of methyl eicosapentaenoate: (A) and (B) same as for Fig. 1.

lapping absorption of the various isomers produced in the alkali-isomerization.

The following principal maxima have been reported for the various conjugated unsaturated acids (12).

Conjugated acid	Wavelength of principal maxima, mμ
Dienoic	224, 232,[a] 238
Trienoic	258, 268,[a] 279
Tetraenoic	288, 301, 315[a]
Pentaenoic	315, 328, 346[a]
Hexaenoic	333, 352, 374[a]

[a] Wavelength generally used in analysis.

The extent of conversion of non-conjugated to conjugated acids and the ratio of the various conjugated isomers produced by alkali-isomerization are dependent upon the conditions used, hence the extinction coefficients (absorptivity) will also vary accordingly. The time and temperature, strength of alkali, solvent used in alkaline reagent, and even the size of sample are important factors in the isomerization and should be kept within the narrow limits specified for the particular method by which the absorptivity constants of the pure reference acids were established.

There are several conditions (methods) of alkali-isomerization now in general use and for which absorptivity constants are available for pure linoleic, linolenic, and arachidonic acids: (A) 6.5% KOH-glycol (5, 38); (B) 11% KOH-glycerol (5), and (C) 21% KOH--glycol (13, 14). Constants are available also for pentaenoic acids in (B) and (C) and for hexaenoic acid in (C) (12). The absorptivity constants for the various acids which are used in deriving the equations for calculation of composition are shown in Table 1.

TABLE I

Specific Extinction Coefficients of Pure Natural Polyunsaturated Acids

Acid	Wavelength	Specific extinction coefficients		
		6.5% KOH-Glycol[a]	11% KOH-Glycerol[b]	21% KOH-Glycol[c]
Linoleic	233	92.1	93.9	91.6
Linolenic	233	61.6	58.6	47.5
	268	50.7	48.6	90.5
Arachidonic	233	56.9	55.0	39.7
	268	52.8	46.8	48.2
	315	21.5	20.3	60.6
C20-Pentaenoic	233	.....	48.9	39.4
	268	.....	33.3	41.2
	315	.....	26.8	82.4
	346	.....	15.0	87.5
C22-Pentaenoic	233	.....	50.0	43.5
	268	.....	35.2	46.0
	315	.....	23.8	56.9
	346	.....	10.9	50.4
C22-Hexaenoic	233	.....	.....	41.7
	268	.....	.....	52.2
	315	.....	.....	29.6
	346	.....	.....	27.7
	374	.....	.....	29.3

[a] 25 minutes isomerization at 180°C. under nitrogen.
[b] 45 minutes isomerization at 180°C. under nitrogen.
[c] 15 minutes isomerization at 180°C. under nitrogen.

Equations for calculation of polyunsaturated acid content of fats, based on the specific extinction coefficients of the pure acids for each alkali-isomerization method, have been published (5, 12, 14, 38). The general method of deriving the equations is illustrated by the following:

1. $k = D/C \times L$, where k = specific extinction coefficient (absorptivity), D = spectra density (absorbance), C = concentration of the solute in grams per liter, and L = length of the optical path through solution in centimeters.

If we assume a specific case, where the sample after isomerization showed absorption maxima at 233, 268, and 315 mμ, but none at higher wavelengths, the basic equations would be 2/, 3/, and 4/, where x = % arachidonic acid, y = % linolenic acid, z = % linoleic acid, k'_{233}, k'_{268}, k'_{315} = specific extinction coefficients of isomerized sample at the wavelengths indicated and (K^4_{233}, K^4_{268}, K^4_{315}), (K^3_{233}, K^3_{268}), and K^2_{233}) = specific extinction coefficients determined on pure arachidonic, linolenic, and linoleic acids respectively.

2. Then $100\,k'_{315} = xK^4_{315}$; or $x = \dfrac{100k'_{315}}{K^4_{315}}$

3. $$100\,k'_{268} = xK^4_{268} + yK^3_{268}; \text{ or } y = \frac{100k'_{268} - xK^4_{268}}{K^3_{268}}$$

4. $$100\,k'_{233} = xK^4_{233} + yK^3_{233} + zK^2_{233};\ \text{or}$$
$$z = \frac{100k_{233} - (yK^3_{233} + xK^4_{233})}{K^2_{233}}$$

Numerical values are substituted for K values (Table I) for the particular method employed and, in the solution of equations x, y, and z, then become expressed in terms of k'_{233}, k'_{268}, and k'_{315}, values measured on the isomerized sample.

Minor corrections for background absorption and for the carboxyl or ester group can be applied to the k′ values under certain conditions, and also approximate corrections can be made for initial (pre-formed) conjugation in the sample according to Brice *et al.* (5).

The percentage of oleic acid can be estimated from the total unsaturation (iodine value) after correction for the polyunsaturated present

$$\frac{100\,I_s - (I_4z + I_3y + I_2x)}{I_1} = \%\ \text{oleic acid}$$

where I_s = iodine number of the sample and I_4, I_3, I_2, and I_1 = theory iodine values of arachidonic, linolenic, linoleic, and oleic acids, respectively. The results would be expressed as percentage acids in the sample. The saturated acids can be estimated by difference: $\%\ S = 100/f - (z + y + x + w)$; $f = 1$ if the sample is fatty acids, 1.05 if methyl esters, and 1.045 if glycerides. This method of estimating oleic and saturated acids may be subject to considerable error depending on the amount of lower homologues of oleic acid present in the sample. For many of our common fats and oils however the method appears to give satisfactory values as judged by the agreement between calculated and observed iodine values (see Table II). Comparison of analyses by the three methods of isomerization is shown in Table III.

Certain oils, such as fish and fish liver oils, contain a very complex mixture of polyunsaturated acids, some of which differ from those of our common fats and oils and for which no extinction coefficients have been determined. Hence the spectrophotometric methods are not applicable in a quantitative sense to such oils although useful qualitative information concerning the nature of the polyunsaturated acids can be gained from the spectral curves.

The methods do not apply to hydrogenated vegetable shortenings because of geometrical isomerization, which takes place during the catalytic treatment. The K-values for the method are based on acids having all-*cis* configuration. Polyunsaturated acids of the *cis-trans* or *trans-trans* type have much slower rates of isomerization and therefore would have different K-values under the conditions of isomerization employed in the method. A modification of the spectrophotometric method has been developed for special application to oils such as tung oil, which contain known conjugated triene acids (α- and β-eleostearic acid) and linoleic acid (35, 36).

For most complete information on the fatty acid composition of fats, particularly those containing a series of saturated and unsaturated acid homologues, it is desirable to prepare the methyl esters of the mixed acids and separate them into a number of relatively simple fractions, preferably according to chain length. The composition of each fraction may then be determined from iodine and saponification numbers, and spectrophotometric analyses. With this information and the weights of the fractions, the fatty acid composition of the original oil can be calculated. Fractional distillation is the more commonly used means of separating the crude esters into simpler fractions. Various types of distillation equipment suitable for the purpose have been described by Carney (8).

The application of chromatographic techniques for fractionation of fatty acids and related substances has been reviewed by Holman (18). Brimley and Barrett (6) have discussed more general applications of chromotography, including descriptions of the various types of techniques employed. Holman pointed out that at the present stage of development chromatography is largely a research tool rather than a routine or standardized method in oil and fat chemistry. Owing to the infinite variety of experimental systems inherent in the empirical nature of adsorption fractionation, much of the work done has been exploratory and unsystematic. Nevertheless numerous examples have been described where one or more of the various techniques have been successfully employed in specific problems to separate closely related substances differing by one or more carbon atoms, in

TABLE II

Analysis of Common Fats and Oil by Spectrophotometric Methods [a] A and B (5)

Results Are Reported as Per cent Acid in Sample

Sample	Method [a]	Conj. Diene	Linoleic	Linolenic	Arachidonic	Oleic [b]	Saturated [c]	Iodine Number	
								Calc.	Obs.
		%	%	%	%	%	%		
Cottonseed Oil	A	0.43	49.8	0.0	0.0	20.4	25.5	108.4	107.8
	B	.....	48.8	0.0	0.0	21.4		107.5	
Soybean Oil	A	0.19	50.7	8.5	0.0	23.0	13.6	135.6	135.2
	B	.....	50.6	8.6	0.0	22.9		135.8	
Linseed Oil	A	0.31	14.4	49.7	0.0	22.6	9.0	182.3	181.8
	B	.....	15.3	49.4	0.0	22.0		182.7	
Lard	A	0.22	10.2	0.68	0.30	49.4	34.9	66.1	66.6
	B	0.18	11.4	0.74	0.35	48.2		67.4	
Tallow	A	0.57	1.18	0.50	0.06	45.6	47.8	45.7	43.9
	B	0.56	1.31	0.55	0.09	45.4		46.0	

[a] A—Isomerization in 6.5% KOH-glycol at 180°C. for 25′ under N_2. B—Isomerization in 11% KOH-glycerol at 180°C. for 45′ under N_2.
[b] Calculated by difference.
[c] Determined by independent method.

TABLE III

Analyses of Common Fats and Oils by Spectrophotometric Methods[a] (A), (B), and (C) (14)

Sample	Method	Sample wt. mg.	Linoleic %	Linolenic %	Arachidonic %	Pentaenoic[b] %
Cottonseed Oil	A	100	52.4	....	....	....
	C	10	52.8	....	....	....
Soybean Oil	A	100	52.3	8.3	....	....
	C	10	51.6	8.0	....	....
Linseed Oil	A	100	16.4	50.6	....	....
	C	10	15.9	51.0	....	....
Lard[c]	B	100	11.3	0.91	0.28	....
	C	10	11.0	0.83	0.30	0.09
Me ester concentrate from lard[c]	B	100	12.3	36.2	14.1	8.4
	C	10	12.3	36.7	15.8	7.5

[a] A—isomerization in 6.5% KOH-glycol at 180°C. for 25′ under N_2.
B—isomerized in 11.5% KOH-glycerol at 180°C. for 45′ under N_2.
C—isomerized in 21% KOH-glycol at 180°C. for 15′ under N_2.
[b] Calculation of pentaenoic acids based on mixtures of C_{20} and C_{22} acids (14).
[c] The same lard is represented in the 2 samples; the me esters were fractionated by low temperature crystallization and by adsorption separation on silicic acid columns to obtain fraction enriched in polyunsaturates.

degree of unsaturation, in positional and geometrical isomerism, by functional group, or by branching of the carbon chain. In some instances, separations were achieved which were hardly possible by other known methods. With systematic investigation, chromatography offers considerable promise of becoming a popular and powerful analytical method in fat chemistry.

Infra-red Spectroscopy. The use of infra-red absorption spectroscopy as an analytical tool in composition studies on fats and oils may be considered as still in the development stage although rapid progress is being made in this field. Wheeler (53) quite recently discussed the principles of infra-red spectroscopy and reviewed the pertinent literature on its application to oils and fats. The absorption of compounds in the infra-red is associated to a large extent with the frequency of vibrations of the atoms of the molecules while absorption in the ultra-violet and visible regions is largely related to frequencies of oscillations of the outer electrons of the atoms. Most of the assignments of wavelengths of absorption bands (maxima) to specific interatomic bonds or structures has been made by the empirical method of comparing the infra-red spectra of a number of pure reference compounds having the specific structure in common. By this procedure many types of chemical bonds have been characterized by definite wavelengths of infra-red absorption. Many other absorption bands characteristic of the over-all structure of the molecule are also usually observed. Therefore absorption spectroscopy in the infra-red region is valuable in helping to establish the structure and identity of unknown compounds. It will, of course, become more useful as further correlations are developed between structures and their infra-red absorption characteristics.

At the present state of development the principal applications of infra-red spectroscopy in composition studies of fats and oils have been largely limited to detection and estimation of geometrical isomers of unsaturated acids and to qualitative identification and approximate estimations of various oxygenated groups formed during oxidation and drying of oils. Rao and Daubert (44) isolated vaccenic acid from beef tallow and confirmed its *trans* configuration by comparing its infra-red absorption with that of elaidic and oleic acid. The vaccenic and elaidic acids showed a strong absorption maximum at 10.25 μ whereas oleic acid showed no evidence of an absorption peak at this wavelength. Similarly, synthetic vaccenic acid (1, 54) proved to have the *trans* configuration.

Shreve, Heether, Knight, and Swern (50) developed a quantitative method, based on differences in absorption at 10.36 μ, for determining trans-octadecenoic acids, esters (including glycerides), and alcohols in the presence of corresponding cis and saturated compounds. The method was compared with the lead-salt-alcohol method for determining trans octadecenoic acids in mixtures with cis isomers (51, 20). The infra-red method was more rapid, specific, and accurate. It has also proved to be a useful tool in following the development of *trans* isomers during hydrogenation (10, 11, 28, 45) and during autoxidation (27).

Infra-red spectroscopy has also given valuable information in studies of *cis, trans* isomers of conjugated unsaturated acids (4, 21, 41); β-eleostearic was shown to be all-trans while α-eleostearic had *cis*-9, *trans*-11, *trans*-13 configuration. Pseudoeleastearic acid was considered to have the all-*trans* structure with the double bonds in the 10, 12, 14 positions.

Glyceride Composition

Methods for determining glyceride composition of fats and oils are important not only because of the fundamental information gained concerning the pattern of glyceride distribution elaborated by plants and animals but also because the physical character and end-use performance of fats are direcly related to their glyceride composition. Hence knowiedge of glyceride composition is valuable in connection with research aimed at improvement of the fat-product for specific uses. Owing to the extremely complex nature of most fats and the difficulties inherent in separations of individual glycerides, methods of analysis

TABLE IV

Comparison of Glyceride Compositions (% mol.) Determined by Oxidation and Crystallization Methods with Values Calculated by Random Distribution Hypotheses (29)

	Oxidation					Crystallization				
	Sm[a]	GS_3	GS_2U	GSU_2	GU_3	Sm[a]	GS_3	GS_2U	GSU_2	GU_3
Lard										
Experimental	38.8	2.8	24.6	58.9	13.7	39.4	2.8	27.4	54.8	15.0
Random	.....	5.8	27.7	43.6	22.9	.....	6.1	28.3	43.4	22.2
Chicken Fat										
Experimental	29.2	2.3	18.3	44.2	35.2	30.4	2.3	17.9	49.2	30.6
Random	.....	2.5	18.1	43.9	35.5	.....	2.8	19.3	44.2	33.7
Palm Oil										
Experimental	51.2	9.4	47.4	30.5	12.7	54.6	9.4	48.1	39.3	3.2
Random	.....	13.4	38.4	36.6	11.6	.....	16.2	40.6	33.8	9.4
Cottonseed										
Experimental	24.6	0.0	13.0	47.7	39.3	26.7	0.0	14.5	51.0	34.5
Random	.....	1.5	13.7	42.0	42.8	.....	1.9	15.7	43.0	39.4

[a] % (mol.) of saturated acid.

have been limited principally to determinations of the four main classes or types of glycerides, *i.e.*, GS_3, GS_2U, GSU_2, and GU_3, where g = glyceryl radical and S and U = saturated and unsaturated acid radicals irrespective of their specific identity. Two methods for determining glyceride composition have been developed, neither of which would be considered of high precision.

Oxidation Method. The first application of oxidation methods to the study of glyceride composition of fats was made by Hilditch and Lea (15). They described a method for determining tri-saturated glycerides (GS_3) by oxidation of the fat in acetone solution with powdered permanganate, followed by aqueous potassium carbonate washes which remove the acidic azelaoglycerides from the unchanged GS_3. The latter is determined by direct weighing. Attempts to extend the oxidation method for the determination of GS_2U, GSU_2, and GU_3 by means of fractionation of the azelaoglycerides were unsuccessful, owing partly to hydrolysis of these products during the oxidation (9, 17, 22). Kartha (22, 23, 24) however showed that hydrolysis can be prevented by maintaining a slight excess of acetic acid during the oxidation with permanganate. The azelaoglycerides can then be separated into two fractions after conversion to their magnesium salts: a) an insoluble fraction containing unchanged GS_3, all the GS_2A[2] and part of the GSA_2 (as their magnesium salts); and b) a soluble fraction comprising the remainder of GSA_2 and all of the GA_3. From the weight of the azelaoglycerides in the insoluble fraction (after acidification and recovery of the product) the saturated acid content, neutralization number of the saturated acids, and the amount of GS_3, determined independently, the amount of GS_2U and GSU_2 can be estimated. The remaining GSU_2 is estimated from the saturated acid content of the soluble fraction (b). The amount of GU_3 is obtained by difference. Typical data obtained by this method are included in Table IV.

Crystallization Method. Systematic fractional crystallization from acetone at several low temperatures, and analyses of fractions produced have been employed (16, 43, 46) as a basis for estimating the distribution of the principal classes of glycerides. Crystallizations in a much simpler way have also been used to determine only the tri-saturated glycerides (30). An outline of a typical system of crystallizations is shown in Figure 5.

From the weights, saponification and iodine numbers, and either total saturated or total unsaturated acid determination, the glyceride composition of each fraction can be estimated and finally the totals for each type calculated as percentage or percentage mol. in the original fat. Sources of possible errors in either method are discussed (29).

Examples of glyceride analyses of four dissimilar fats and oils by both methods are shown in Table IV along with values calculated on the basis of random distribution.

Neither of these methods for determining glyceride composition however takes into account the actual position a given acid occupies on the glyceride molecule. Quimby *et al.* (43) presented evidence, based on x-ray diffraction and thermal techniques, which indicates that the palmityl group is predominantly in the 2-position in lard whereas in beef and mutton tallow it is

[2] GS_2A, GSA_2 and GA_3 = disaturated monoazelao-, monosaturated diazelao-, and triazelao-glycerides.

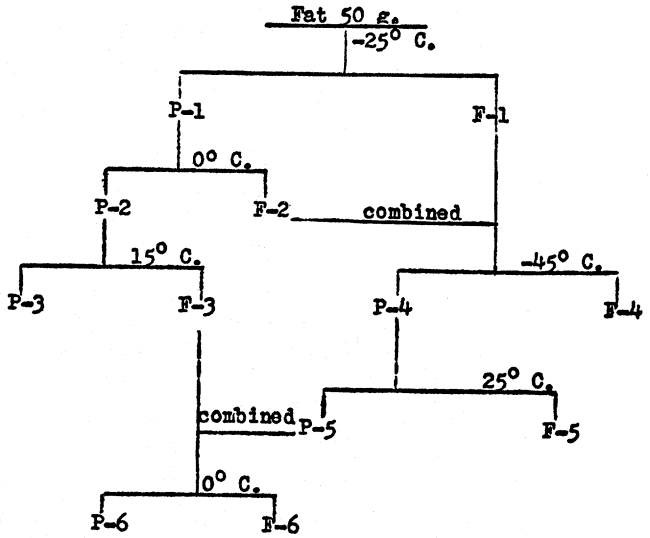

FIG. 5. Steps in the fractional crystallization of fats from acetone (30 ml./g.).

largely in the 1-position. Hence they concluded that the distribution is not random in character. In true random arrangement the acyl groups would occupy the 1- and 2-positions indiscriminately or strictly according to chance.

In conclusion, it may be said that remarkable progress has been made in the past decade in the development of relatively simple, rapid, and accurate methods for determining fatty acid composition of fats and oils, principally by means of ultraviolet spectrophotometry. More work is required on the extension of the methods to highly complex fats which contain unsaturated acids of 2, 3, 4, 5, and 6 double bonds.

Infra-red spectroscopy, although quite recently applied to composition studies of fats, already has provided valuable information on the geometrical configuration of unsaturated acids and is a convenient means of following changes in configuration when the fatty materials are subjected to treatments, such as selective hydrogenation, catalytic isomerization, and oxidation. It is valuable in establishing the structure and identity of compounds and the presence or absence of certain types of oxygen containing groups such as may be formed during oxidation.

Chromatographic techniques offer considerable promise, through systematic study, of becoming a very valuable analytical tool as well as means of fractionation for determining composition of fatty materials.

The methods available for determining glyceride distribution in fats should be supplemented with information based on thermal techniques and x-ray diffraction.

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